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High rates of polygyny in tropical Mexico within the native range of Vespula squamosa.

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Abstract

Polygyny, or the formation of colonies with multiple cooperating queens, has been observed in a variety of social Hymenoptera and likely exists as a convergent evolutionary strategy. Polygyne cooperation has been observed in several *Vespula* sp. and is correlated with a perennial social strategy. This perennial-polygyne behavior has been observed most commonly within the tropical and subtropical regions of the invasive *Vespula pensylvanica* and *V. germanica*, and rarely within their native temperate ranges. This phenomenon has been relatively undocumented within the tropical portions the *V. squamosa* native range, despite it being observed in their temperate ranges several times. We observed polygyny in seven out of eight colonies of *V. squamosa* at a Santiago Apoala site in Oaxaca, Mexico. Our findings suggest that polygyny in these *Vespula* species is not solely the product of a genetic or population bottleneck resulting from introduction, but rather some undetermined environmental effects.

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Introduction

Polygyny is the act of multiple reproductive queens, or gynes, cooperating within a singular colony. Many different forms of polygyny exist, each working with a varying number of queens. Polygyne social structures have been observed across Hymenoptera and are likely the result of multiple convergent evolutionary pathways (Boomsma et al, 2014; Ratnieks et al, 1996). Polygynic behavior often works in a tradeoff, as the polygyne colonies weaken the strength of kin selection pressures in exchange for the added benefits of a larger nest and population that are possible with the extra reproductive power, as well as additional diversity within the colony (Hughes et al, 2008; Ratnieks et al, 1996; Ross & Matthews, 1982). Another major social change that results in colonial diversity is polyandry in which a singular queen will meet with multiple males. Polyandry and polygyny are usually exclusive to one another as polgynyous species usually have a lower mating frequency, while polyandrous species usually do not form polygynous colonies (Hughes et al, 2008).

Vespula wasps, frequently referred to as yellowjackets, are located primarily in North America, Europe, and Asia. Compared to their sister genus *Dolichovespula*, they tend to nest at a higher rate underground. In general, most *Vespula* sp. perform an annual colony cycle in which a single queen will perform the typical nest construction in spring and maintain the colony until fall. At this point the workers usually die out and the daughter queens usually overwinter in or near the nest before dispersing in the spring to start anew. There are some notable exceptions to this strategy; *V. squamosa, V. pensylvanica,* and *V. germanica,* in which perennial strategy can sometimes occur. In this perennial strategy multiple of the queen's reproductive offspring will overwinter and stay in the nest and maintain a reduced worker population, before the cooperating queens cause the nest population to explode in the spring. Polygyny seems to be linked to perenniality in *Vespula*, as polygyny has been observed commonly in perennial colonies (Goodisman, 2001; Landolt, 2009; Ratnieks, 1996; Ross, 1982). This is likely due to the fact that in perennial colonies the mother queen does not usually survive longer than a year. Polygyny is likely a requirement for perenniality, but polygyny is not reliant on perenniality for its formation. For example, some *V. pensylvanica* within their introduced Hawaii range have polygyne nests that aren't perennial (Hanna et al, 2013; Loope et al, 2021).

In *Vespula*, polygyny has been observed at a higher rate in introduced tropical and subtropical populations (Goodisman, 2001; Hanna et al, 2013). There is still speculation on what factors influence introduced tropical and subtropical polygyny, as it could be influenced by the relatively larger amount of

resources available in these ranges allowing for an increased selective pressure towards cooperation and resource sharing. Alternatively, it could be the result of an overall warmer climate allowing for the non-queen nestmates to survive winter more easily or the effects of a population or genetic bottleneck that results from the introduction. In the case of a population or genetic bottleneck, a particular mutation that encourages polygyny that is uncommon in the native population could be present in the individual that was introduced and thus spread (Tsutsui et al, 2000). This could result in a loss of recognition cues that non-nestmates use to distinguish between one another. This polygyne behavior resulting from a population or genetic bottleneck has been observed in several species of ants including *Linepithema humile* and *Solenopsis invicta* that become invasive and often form unicolonies within their invasive ranges (Giraud & Keller, 2002; Lofgren & Williams, 1984). Polygyny has been observed facultatively in several species, the southern yellowjacket, *V. squamosa,* and the western yellowjacket, *V. pensylvanica,* and the german yellowjacket, *V. germanica* (Goodisman, 2001; Ratnieks et al, 1996; Ross & Matthews, 1982). Each of these species have been observed constructing massive polygyne nests that can house over 100,000 individuals. In *V. pensylvanica* these nests have been seen commonly in the invasive portions of their Hawaii range and on rare occasions within their California home range (Akre & Reed, 1981; Gambino 1991; Ratnieks et al, 1996). Similar to *V. pensylvanica*, *V. germanica* has also made polygynous nests in the invasive portions of their range in New Zealand and Australia, however no polygynous nests have been found within their native range (Goodisman, 2001).

V. squamosa is an interesting corner case as they are not currently invasive in any location and there is relatively little known about them within their tropical range. Like *V. pensylvanica*, *V. squamosa* also has had perennial-polygyne nests found at rarer quantities in more temperate regions like Georgia and Florida.

V. squamosa is a known facultative temporary social parasite of their host species *V. maculifrons* (Akre et al, 1981; Hoffman et al, 2008). Having a rather large range stretching from Pennsylvania down to Central America, *V. squamosa* tends to prefer distrubed or disrupted habitats free of dense roots and other vegetation (Akre et al, 1981). *V. squamosa*, similar to *V. pensylvanica* and *V. germanica*, is known to have two overwintering strategies; annual and perennial (Akre & Reed, 1981; Goodisman, 2001; Ross 1982).

This perennial-polygyne strategy has only been observed in the southern, and consequently more tropical, portions of native *V. squamosa* range. Our aim was to determine the extent to which polygyny occurs at a site in southern Mexico through the use of genetic parental analysis of offspring workers to act as a comparison between introduced tropical and subtropical populations and a native population from a similar climate.

Materials and Methods

Collection and Study Site

V. squamosa gynes, males, and workers were collected from 8 colonies arbitrarily labeled 1 through 8 in Santiago Apoala in Oaxaca Mexico from November 21st 2014 to December 6th 2014 by Nathan Derstine, Sebastien Jimenez and Gerhard and Regine Gries. These colonies were found in disturbed

farmland habitats. Individual yellowjackets were sorted by sex, caste, and colony origin. 48 workers from each colony, with the exception of colonies #1, #2, and #4, were selected for DNA extraction and PCR analysis. Colonies #1, #2, and #4 contained fewer workers so 33, 5, and 4 workers were selected respectively. Additionally, 4 males were selected from nest #2.

DNA Extraction, PCR Amplification, and Genotyping

DNA extraction was performed on the selected yellowjackets. An antennae was selected from each individual and placed in a 10% Chelex solution before being incubated at 95°C for 20 minutes. The incubated samples were then frozen in preparation for PCR. Each selected yellowjacket had PCR performed on them at 8 different variable loci with the proceeding primers; List2014, Rufa18, List2013, List 2020, List2003, VMA3, VMA6, and Rufa05 (Hoffman et al, 2008). These primers were derived originally from other related *Vespula* and *Dolichovespula* sp*.* (Daly et al, 2002; Foster et al, 2001; Hasegawa & Takahashi, 2002). The PCR mixings were all performed on ice and 10-15 µL reactions were used. A master mix for each primer was made using either of a GoTaq or GoTaq FLEXI buffer and primer, deoxynucleoside triphosphate (dNTP), a forward and reverse primer corresponding to the desired loci, a dye-labeled tag (Schuelke, 2000), molecular-grade water, and a variable amount of a magnesium catalyst was sometimes added that was dependent on the loci primer and whether GoTaq FLEXI was used . The master mix to DNA ratio was between 90-10% and 85-15%. The DNA ratios were increased in several loci in order to better improve the odds of getting a successful amplification procedure. Amplification was

performed using a PCR cycle that would preheat the machine to 95°C. Once the samples were inserted, the machine was then set to run for 2 minutes at that temperature before running 40 cycles of 95°C for 30 seconds, a variable annealing temperature based on the selected primer between 48°C and 60°C for 30 seconds and then a 72°C for 30 seconds. Several samples had to be redone at different temperatures after failing at their original annealing temperatures. After cycling, the sample was incubated for 5 minutes at 72°C, before eventually being cooled to room temperature.

Microsatellite Loci Scoring and Software

The samples, once amplified, were then analyzed through an ABI 3500 sequencer using an orange dye-labelled size standard (MCLabs) before being sorted and proofread by two individuals through the use of GeneMarker. The allelic distributions of each individual were taken and placed into an Excel spreadsheet for compilation. As a means of creating matrilineages, the genotypes determined by GeneMarker were taken for each individual and then run through two statistical analysis engines called COLONY and MateSoft. In order for the data to be processed optimally, the four males collected from nest #2 were excluded from COLONY analysis. COLONY parameters were set to process a dioecious haplodiploid species set to isolate individuals by nest in order to prevent non-nestmate siblingship recognition using the Full-likelihood method at the selected eight loci for 286 individuals in a “Very Long Run” with an expected error rate for each locus of 0.05. Additionally, COLONY settings were adjusted to account for the multiple mating of females but not males. COLONY

was able to produce both the most likely assortment of possible queens as well as their suggested male mates per nest based on assessing allele frequencies and sorting of their observed offsprings' genotypes. (Jones et al, 2010) This data was then sorted to match each individual with their mother. MateSoft v1.0 was used as an independent check for polygyny.

Results

We successfully genotyped 280 individuals. Allelic distributions of the genotyped individuals were determined through the use of GeneMarker (Figure 1) before being used for COLONY and MateSoft analysis. COLONY analysis shows that there are roughly 44 minimum unique queens total and that each colony had 3 or more minimum unique queens (Figure 2). Polygyny and polyandry were observed in each nest with variance in the overall number of queens in each nest. Each nest averaged 5.5 minimum unique queens. The overall highest number of minimum unique queens was from nest #3 with a total of 11. MateSoft crosscheckng shows that obligate polygyny was observed from every nest excepting #2 from at least one or more loci (Table 1). This potentially conflicts with the results received from previous COLONY output as 3 unique queens were observed within nest #2 (Tables 2 & 3).

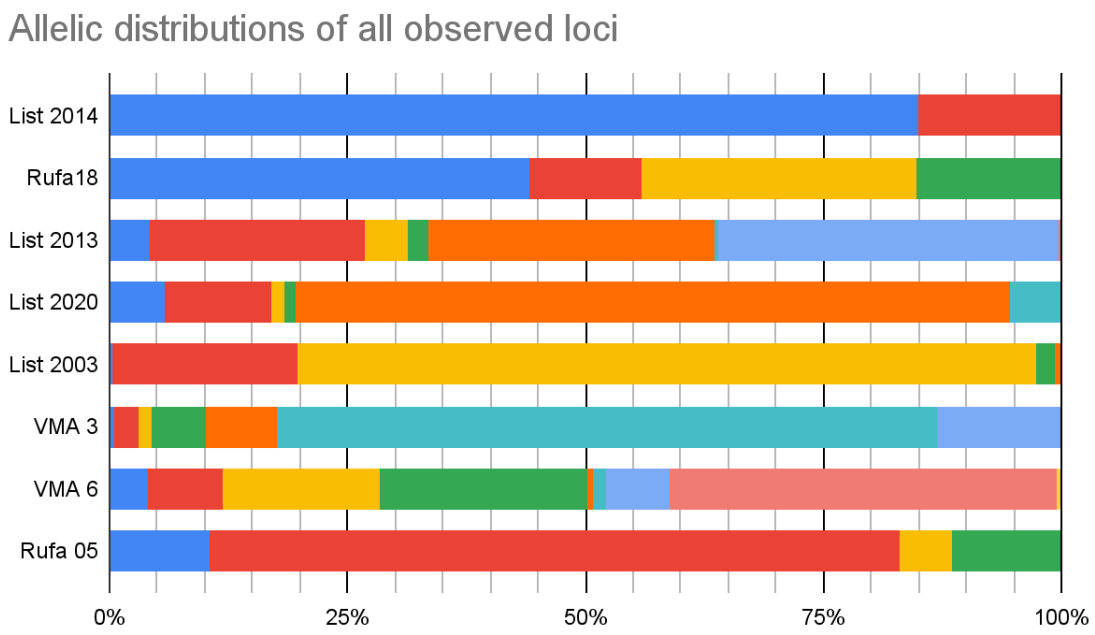

Figure 1. Frequency of allele distributions for each of the eight observed loci. Each colored portion indicates a percentage of a different allele that makes up the locus listed.

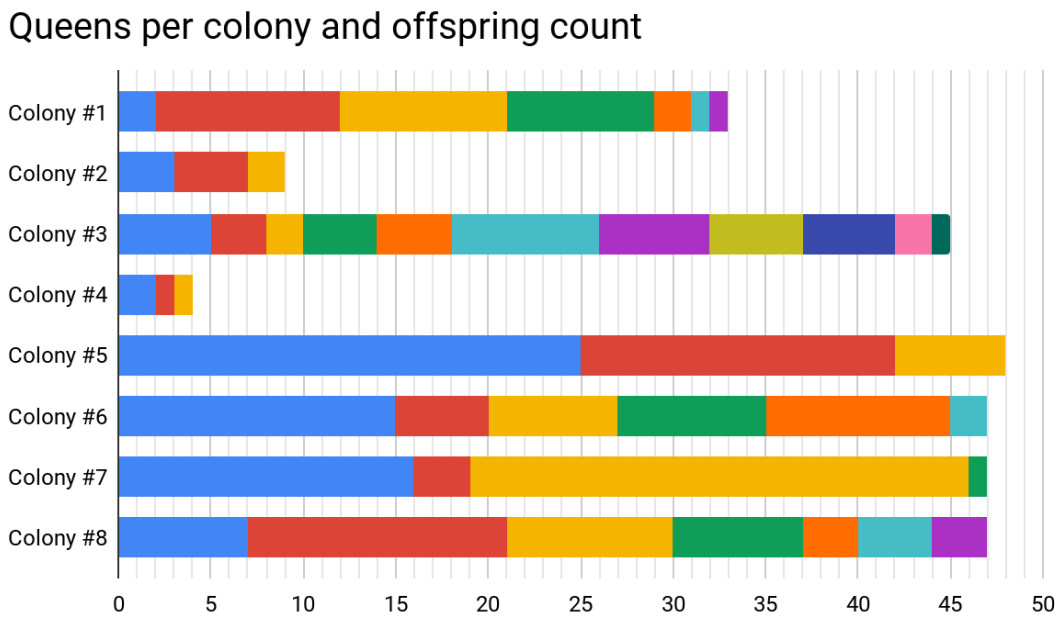

Figure 2. Queens found in each colony and the number of offspring they are related to as determined by COLONY. Different colors represent unique queens within each colony.

	Colony #1	Colony #2	Colony #3	Colony #4	Colony #5	Colony #6	Colony #7	Colony #8
List 2014	Less than .0001	Error	0.03469	0.625	Less than .0001	Less than .0001	Less than .0001	Less than .0001
Rufa 18	0	1	0	1	0	0	0.005925	0
List 2013	0	1	0	1	Less than .0001	0	0.04356	0
List 2020	Less than .0001	0.625	Less than .0001	1	Error	Less than .0001	0.7709	Less than .0001
List 2003	0.00149	0.375	Error	0.625	1	Less than .0001	Less than .0001	Less than .0001
VMA3	0	1	Less than .0001	Error	Less than .0001	0	Error	Less than .0001
VMA6	0.007197	1	0	0	Less than .0001	0	0	0
Rufa 05	0	0.625	Less than .0001	0.625	Less than .0001	Less than .0001	0.8679	Less than .0001

Table 1. Matesoft output probabilities. All values are proportions of the likelihood of true polygyny occurring within each colony at each locus with values closer to 0 indicate a higher likelihood of polygyny and values closer to 1 indicate a higher likelihood of monogyny. Values at 0 indicate obligate polygyny. Matesoft was unable to process some loci at certain values.

	Fathers	Males present at collection	Queens	Queens present at collection
Colony #1	18	Yes	7	No
Colony #2	4	Yes	3	Yes
Colony #3	24	Yes	11	Yes
Colony #4	4	Yes	3	Yes
Colony #5	12	Yes	3	Yes
Colony #6	22	Yes	6	Yes
Colony #7	9	Yes	4	No
Colony #8	19	Yes	7	Yes

	Analyzed workers	Loci with monogyny <1%	Succesfully genotyped individuals
Colony #1	33	8	33
Colony #2	9	0	9
Colony #3	48	6	45
Colony #4	4	1	4
Colony #5	48	6	48
Colony #6	48	8	47
Colony #7	48	4	47
Colony #8	48	8	47

Tables 2 & 3. Number of males, number of queens, number of analyzed offspring, number of loci per colony with 1% or lower likelihood of being monogynous, and successfully genotyped individuals per nest.

Discussion

Polygyny appears to be a relativley common occurrence in the Santiago Apoala range of *V. squamosa*. Polygyny was observed in all or nearly all nests we observed. The rate at which polygyny occurs within the Santiago Apoala range has not been seen in other analyzed parts of the native range of *V. squamosa* (Hoffman et al, 2008). Other colonies have been observed performing this polygyne strategy at a similar degree in nearby Guatemala, suggesting that

this could be a common occurrence in other areas nearby (Landolt et al, 2009). The presence of polygyny within the native range of *V. squamosa* suggests that *V. squamosa* queens throughout their range are likely capable of performing this perennial-polygyne strategy but there are some regional or climate factors that encourage this strategy within the observed area. These factors could be related to an abundance of resources that would be present in the tropical portion of their range that could cause a selective advantage for cooperation and resource sharing. In this case, an increase in resources could encourage an increase in colony size, and by extension overall colony fitness, that a lone queen could not reasonably sustain. Alternatively, an increase in winter temperatures such that the whole colony can overwinter or some other environmental influence may be responsible.

The observation of polygyny at relatively high rates in *V. squamosa* within their native range suggests that genetic or population based bottlenecking is at least not the sole cause of polygyny in other tropical and subtropical polygynes that are invasive like *V. pensylvanica* and *V. germanica*. Rather, their behavior is the result of some other factor that could be related to climate or regional differences that encourages polygyny in their invasive range. Whether or not polygyny being a result of environmental factors as opposed to genetic ones could be applied to non-*Vespula* species is unclear.

The variance in offspring allele distributions could be high enough to support that daughters of multiple unique queen lineages exist in the same nests, however it also could be the result of polyandry. There are several ways in which

queen joining could occur. This may be the result of potentially foreign queens joining the nest to perform cooperative grouping. Alternatively, joining could be caused by daughter queens being recruited into the nest they originated from (Goodisman et al, 2001). Cooperative grouping like this is not unheard of within Vespula (Gambino, 1991). This could be the result of cooperation between the residing queens and foreign queens as a result of a high resource quantity in the local area. As an unlikely alternative, multiple queens could gather together and form a single nest all at one time. While this could be feasible in polygyne *V. squamosa* colonies it has not been observed in the temperate range where they have been most studied and is unlikely based on how colonies are formed.

Further efforts could be applied to determining the processes that cause polygynous colonies to form through the frequent observation of monogyne colonies that have a high likelihood of becoming polygyne. Observation of polygyne precursors could explain what environmental criteria are most impactful for the formation of polygyne colonies as well as provide information of behavioral or physiological shifts that may occur in the transition between monogyne and polygyne.

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